

37 °C with gentle tapping,

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- 3) mixing with an equal volume of Tris-HCl and EDTA buffer saturated phenol,
 - 4) centrifuging at approximately 3,000 rpm for approximately 10 min, and collecting the upper aqueous phase containing DNA,
 - 5) mixing with equal volume of phenol and chloroform,
 - 6) centrifuging at approximately 3,000 rpm for approximately 10 min, and collecting the upper aqueous phase containing DNA,
 - 7) mixing with an equal volume of chloroform/isoamyl alcohol prepared at a ratio of 96:4,
 - 8) centrifuging at approximately 3,000 rpm for approximately 10 min,
 - 9) collecting the upper aqueous phase containing DNA and precipitating using a salt and ethanol,
 - 10) centrifuging at approximately 13,000 rpm for approximately 30 min,
 - 11) removing the upper liquid phase and air drying the bottom DNA pellet,
 - 12) dissolving the air dried DNA pellets in Tris-HCl and EDTA buffer.

C2

4. (Amended) The method of Claim 9 wherein the method further includes the steps of analyzing the biologically active transcription factors present in the top clear supernatant of step 1.

Insert Claim 9 (Newly submitted). Claim 9 which is the same as the **Examiner's proposed Amendment of Claim 2** (previously submitted) except for minor changes as noted in the REMARKS.

9. (Newly submitted) A method for simultaneous isolation of biologically active transcription factors and DNA, wherein no ultracentrifugation or sonication is used, comprising the following steps:

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- a) collect cells from a culture or from a patient,
 - b) washing said cells at least once with PBS,
 - c) suspend and maintain said cells in buffer A (cell lysis buffer) for approximately 15 minutes wherein buffer A comprises: 20 mM Hepes, pH 7.9, 10 mM NaCl, 3 mM MgCl₂, 0.1% NP-40, 10% glycerol, 0.2 mM EDTA, 1 mM DTT, 0.4 mM PMSF, 1 µg/ml antipain, 1 µg/ml leupeptin,
 - d) centrifuge the suspension of step c at approximately 2,000 rpm for approximately 5 min at approximately 4 °C,
 - e) remove the upper cytoplasmic supernatant fraction and then clarify this fraction by adding buffer D (cytoplasmic extraction clarification buffer) at approximately 4 °C, wherein buffer D comprises: 20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 40% glycerol, 1 mM DTT, 0.4 mM PMSF, 1 µg/ml antipain , 1 µg/ml leupeptin,
 - f) centrifuge the clarified fraction formed in step e approximately 13,000 rpm for approximately 15 min,
 - g) remove and freeze the top clear supernatant on dry ice then store the frozen top clear supernatant at approximately -86 °C,
 - h) wash the bottom nuclear fraction formed at the end of step d with buffer B (extraction buffer without salt) wherein buffer B comprises: 20 mM Hepes, pH 7.9, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.4 mM PMSF, 1 µg/ml antipain , 1 µg/ml leupeptin,
 - i) centrifuge the mixture formed in step h at approximately 2,000 rpm for approximately 5

minutes at approximately 4 °C, thereby pelleting cellular nuclei,

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- j) suspend the pelleted nuclei of step i in buffer C (extraction buffer with salt) on ice, tapping the suspended mixture for approximately 45 minutes whereby nuclear proteins are extracted, wherein buffer C comprises: 20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.4 mM PMSF, 1 µg/ml antipain , 1 µg/ml leupeptin,
 - k) centrifuge the mixture formed in step j at approximately 13,000 rpm for approximately 15 minutes, at approximately 4 °C,
 - l) remove the top clear supernatant comprising biologically active transcription factors from the bottom nuclear fraction containing nucleic acids, and quick freeze on dry ice and store at approximately -86°C,
 - m) extract DNA from said bottom nuclear fraction.

Insert Claims 10 and 11 (Newly submitted).

10. (Newly submitted) The method of Claim 9 wherein the method further includes: the steps of analyzing the biologically active cytoplasmic factors present in the top clear supernatant of step g.

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11. (Newly submitted) The method of Claim 9 wherein the step l, as proposed by the Examiner, is followed by the step of: , and quick freeze on dry ice and store at approximately -86°C.

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